

CLAIMS

1. A megakaryocyte differentiation factor having the following properties;

(1) stimulating differentiation of megakaryocytes;

(2) exhibiting a molecular weight of 55 to 57 kD as determined by gel filtration and SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and having no intermolecular disulfide linkage;

(3) exhibiting an isoelectric point of  $6.5 \pm 0.5$ ; and

(4) having at least one of the amino acid sequences shown in SEQ ID NO: 1 to 9 in the Sequence Listing.

2. A megakaryocyte differentiation factor according to claim 1, produced by human cells.

3. A megakaryocyte differentiation factor according to claim 2, produced by human cancer cells.

4. A megakaryocyte differentiation factor according to claim 3, produced by cells derived from human epidermoid carcinoma cell A431.

5. A megakaryocyte differentiation factor according to claim 4, produced by cells derived from human epidermoid carcinoma cell A431, cultured in a protein-free medium.

6. A megakaryocyte differentiation factor having an amino acid sequence the same as the native amino acid sequence of a megakaryocyte differentiation factor according to claim 1, an amino acid sequence wherein one or more than one amino acid residue in said native amino acid sequence is deleted, an amino acid sequence wherein one or more than one amino acid residue in said native amino acid sequence is replaced with other amino acids, an amino acid sequence wherein one or more than one amino acid is added to said native amino acid sequence, or an amino acid sequence including a combination of said amino acid modifications.

7. A megakaryocyte differentiation factor essentially consisting of the amino acid sequence shown in SEQ ID NO: 30.

5 8. A megakaryocyte differentiation factor having an amino acid sequence wherein one or more than one amino acid residue in the amino acid sequence shown in SEQ ID NO: 30 is deleted, an amino acid sequence wherein one or more than one amino acid residue in the amino acid sequence shown in SEQ ID NO: 10 30 is replaced with other amino acids, or an amino acid sequence wherein one or more than one amino acid is added to the amino acid sequence shown in SEQ ID NO: 30 or an amino acid sequence including a combination of said amino acid modification.

15 9. A megakaryocyte differentiation factor according to claim 6, which is glycosylated.

10. A megakaryocyte differentiation factor according to claim 7, which is glycosylated.

20 11. A megakaryocyte differentiation factor according to claim 8, which is glycosylated.

25 12. A megakaryocyte differentiation factor obtainable from a culture of transformed cells constructed by a gene recombination technique using a polynucleotide hybridizable with a polynucleotide coding for at least one of the amino acid sequences shown in SEQ ID NO: 1 to 9.

30 13. A megakaryocyte differentiation factor according to claim 6, wherein the N-terminus of the factor is biochemically or chemically modified, preferably the first methionine is deleted and the second alanine is acetylated.

35 14. A megakaryocyte differentiation factor according to claim 8, wherein the N-terminus of the factor is biochemically or chemically modified, preferably the first methionine is deleted and the second alanine is acetylated.

15. A megakaryocyte differentiation factor according to claim 12, wherein the N-terminus of the

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factor is biochemically or chemically modified,  
preferably the first methionine is deleted and the  
second alanine is acetylated.

5       16.    An isolated DNA coding for a megakaryocyte  
differentiation factor according to claim 1.

      17.    An isolated DNA coding for a megakaryocyte  
differentiation factor according to claim 6.

      18.    An expression vector comprising a DNA  
according to claim 16.

10       19.    An expression vector comprising a DNA  
according to claim 17.

      20.    A host transformed with an expression vector  
according to claim 18.

15       21.    A host transformed with an expression vector  
according to claim 19.

      22.    A pharmaceutical composition comprising a  
megakaryocyte differentiation factor according to  
claim 1 and a pharmaceutically acceptable carrier.

20       23.    A pharmaceutical composition comprising a  
megakaryocyte differentiation factor according to  
claim 6 and a pharmaceutically acceptable carrier.

      24.    A pharmaceutical composition according to  
claim 22, for treating thrombo cytopenia.

25       25.    A pharmaceutical composition according to  
claim 23, for treating thrombo cytopenia.

      26.    An antibody to megakaryocyte differentiation  
factor according to claim 1.

      27.    An antibody to megakaryocyte differentiation  
factor according to claim 6.

30       28.    A process for production of a megakaryocyte  
differentiation factor according to claim 1,  
comprising the steps of culturing a host according to  
claim 20, and recovering the megakaryocyte  
differentiation factor.

35       29.    A process according to claim 28, wherein the  
host is silkworm Bombyx mori.

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